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Serum MMP3 and IL1-RA levels may be useful biomarkers for detecting asthma and chronic obstructive pulmonary disease overlap in patients with asthma

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ABSTRACT

Background: Asthma and chronic obstructive pulmonary disease (COPD) overlap (ACO) is characterized by concurrent features of asthma and COPD. Since disease pathogenesis, severities, and treatments differ between asthma and ACO, it is important to differentiate them.

Objective: To clarify and compare the characteristics of ACO and asthma and identify the serum biomarkers for differentiating them, especially in older patients.

Methods: This study used the data of 639 participants from the nationwide cohort study, the NHOM-Asthma study, an asthma registry in Japan, with complete information on smoking history, respiratory function, and serum biomarkers. ACO was defined as the self-reported comorbidity of COPD or emphysema, or with obstructive pulmonary function and smoking history (pack-years≥10). The clinical characteristics of patients with ACO and asthma without COPD were compared. The serum biomarkers for differentiation were examined using receiver operating characteristic curves and multivariable analysis. The associations between the bio-markers and age were also analyzed.

Results: Of the 639 asthma patients, 125 (19.6%) were diagnosed with ACO; these patients were older and male-dominant and had a higher prevalence of comorbidities such as hypertension, diabetes, and stroke. Among the serum biomarkers that were significantly different between ACO and asthma without COPD, the YKL-40/CHI3L1, MMP3, and IL-1RA levels showed a high area under the curve for discriminating ACO. Only the MMP3 and IL-1RA levels were significantly higher among ACO patients, regardless of age and sex; the YKL-40/CHI3L1 levels were not different due to the effect of age.

Conclusion: MMP3 and IL-1RA may be useful serum biomarkers for distinguishing ACO from asthma.

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INTRODUCTION

Asthma is among the most common chronic respiratory diseases in the world, and it can occur at any age.[1-3] In Japan, as a super-aging country,[4] the number of older patients suffering from asthma is growing rapidly. This is becoming concerning because older adults account for the majority of asthma-related deaths.[5] They also have more severe and uncontrolled asthma due to their comorbidities, frailty, and cognitive decline.[6-8]

Chronic obstructive pulmonary disease (COPD) is another common pulmonary disease that is especially prevalent among older people with a history of heavy smoking.[9] Asthma-COPD overlap (ACO), or asthma-COPD overlap syndrome, represents the concurrence of asthma and COPD features, and is more prevalent in older adults.[9,10] Asthma and COPD are characterized by obstructive airflow impairment due to chronic airway inflammation; however, their inflammatory pathogeneses differ. Asthma is typically characterized by inflammation mediated by eosinophils and activated type 2 helper T cells, whereas COPD is predominantly characterized by inflammation mediated by neutrophils and macrophages.[11,12] Therefore, their treatment targets differ. In addition, patients with both asthma and COPD have a greater burden of symptoms, lower quality of life, more rapid decline in lung function, and higher mortality rate than patients with either of them.[1] Therefore, it is important to distinguish patients with asthma, COPD, and ACO to provide the best medical care.

Distinguishing between asthma and ACO can be challenging in clinical practice, particularly in older patients. Therefore, identifying biomarkers that can aid in the differentiation of these conditions is crucial. Given that there are distinct phenotypes of asthma in patients with no or less smoking history, the phenotype of ACO may manifest in distinct characteristics from those of asthma without COPD, presumably with distinct biomarkers.[13,14] Reports on biomarkers for distinguishing ACO from asthma are scarce, especially for older patients with asthma.[15-17] Therefore, this study aimed to compare the characteristics of ACO and asthma in Japan and identify serum biomarkers for distinguishing them, especially in older patients.

METHODS

Study design

This was a cross-sectional, observational study. This study used data obtained from the nationwide cohort study, NHOM-Asthma Study,[18] a prospective asthma registry in Japan, and its protocol was approved by the ethics committee of the Institutional Review Board of the National Hospital Organization, Tokyo National Hospital (Approval No. 529). The NHOM-Asthma study, registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR; UMIN000027776), is a nationwide prospective, multicenter cohort study that investigated the characteristics and phenotypes of adult severe asthma in Japan; the full details of the study design have been published previously.[18] Adult asthma patients (aged ≥18 years) diagnosed and treated for asthma for more than 1 year were recruited between July 2017 and September 2018 from 27 national hospitals (mainly belonging to the National Hospital Organization, NHO) across Japan and treated based on their physicians' standard practices in a real-world clinical setting. Clinical data were obtained from the medical records of the patients, but the NHOM-Asthma study did not obtain airway reversibility test data or CT scans. They were requested to complete the Asthma Control Questionnaire 6, which is a basic questionnaire. In addition, patients filled out questionnaires about their smoking history and any diagnosis of COPD/emphysema, gastroesophageal reflux disease (GERD), sleep apnea syndrome, hypertension, diabetes, heart disease, stroke, arthritis,

osteoporosis, mental disorder, sinusitis, and allergic comorbidities (including allergic rhinitis, allergic conjunctivitis, atopic dermatitis, hay fever, food allergy, drug allergy, and urticaria). In Japan, 38.8% of the population in 2019 experienced Japanese cedar pollinosis.[19,20] Therefore, comorbidities of hay fever, specifically Japanese cedar/cypress/ragweed pollen allergy, and allergic rhinitis, which is caused by allergies other than pollen, were obtained separately. In addition, the frequencies of asthma exacerbation that required systemic corticosteroids and hospital admission in patients who were followed up for 1 year after recruitment were collected.

Serum samples from the patients receiving treatment were collected and frozen at −20 °C at the participating hospitals for as long as 1 year. They were subsequently assessed at the central laboratory for NHOM-Asthma, and the data were used for the present study.
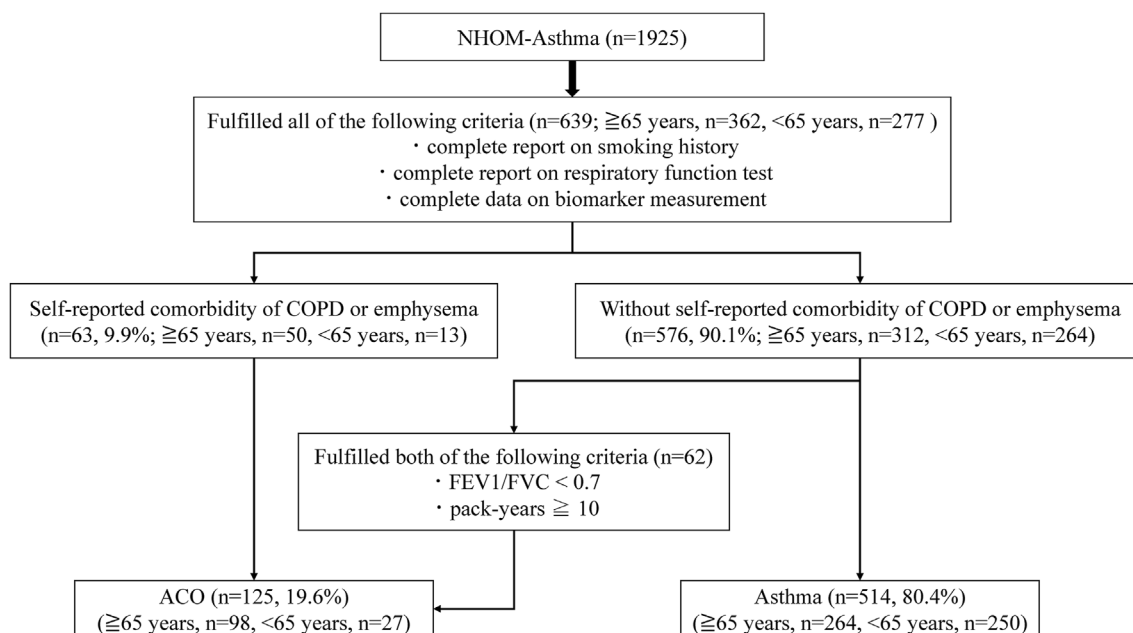
Patients

The full details of the patients enrolled in NHOM-Asthma have been described previously.[18] The requirement for informed consent was waived because this study was only based on the data from the previous study. Of the patients enrolled in NHOM-Asthma, 639 who had complete data

on their smoking history, respiratory function tests, and serum biomarker measurements were first selected for inclusion in the present study (Fig. 1). While there are many studies on ACO, a universal definition has not yet been established. However, information such as smoking history, allergic history, physician diagnosis, and pulmonary function are important factors, and the Global Initiative for Asthma (GINA) recommends that the diagnosis of ACO is based on the presence of persistent airflow limitation and characteristics of asthma and COPD, such as age, smoking history, and common triggers.[21,22] In the present study, the ACO patients were designated in 2 steps: first, patients with self-reported comorbid COPD or emphysema based on the patient-reported questionnaire were designated as ACO. Second, patients who did not self-report comorbid COPD or emphysema but had a smoking history of more than 10 pack-years and showed signs of obstructive respiratory dysfunction, as indicated by a forced expiratory volume in 1 s (FEV1)/forced vital capacity of <0.7, were also designated as ACO. Patients without ACO were classified as asthma patients without COPD.

Assessments and statistical analysis

The baseline characteristics and serum biomarkers of the ACO patients were compared with

Fig. 1 Flowchart for the selection of patients with ACO from the asthma patients enrolled in NHOM-asthma. Abbreviations: ACO, asthma and COPD overlap; COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity

those of asthma patients without COPD. Receiver operating characteristic (ROC) curve analysis was performed for the biomarkers that were significantly different for the 2 groups based on univariate logistic regression. This was used to investigate and compare the usefulness of biomarkers in distinguishing ACO from asthma, and the optimal cut-off points were determined using the Youden Index. Additionally, the levels of candidate serum biomarkers in ACO patients and asthma patients without COPD were compared between older patients ($\geq$65 years) and non-older patients (under 65 years), respectively. The chi-squared test was used to analyze the categorical data, and Wilcoxon's rank sum test was used to analyze the continuous data. Furthermore, a multivariable logistic regression analysis was conducted to examine the effect of age and sex with the presence of ACO as the endpoint. Spearman's correlation coefficients were used to determine the association between the levels of the biomarkers and age and pulmonary function. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using the JMP® Pro software version 16 (SAS Institute Japan, Tokyo, Japan).

RESULTS

Baseline characteristics of ACO and asthma patients without COPD

Of the 639 patients, 125 (19.6%) were diagnosed with ACO based on the criteria used in the present study (Fig. 1). The baseline characteristics of ACO and asthma patients without COPD are shown in Table 1. Compared with the asthma patients without COPD, the ACO patients were significantly older (73 [66–77] vs 65 [50–74], $p < 0.001$) and predominantly males (76.8% vs. 32.5%, $p < 0.001$). ACO patients had lower FEV1, FEV1/forced vital capacity, and %FEV1 than the asthma patients without COPD. Compared with the asthma patients without COPD, the ACO patients more frequently had hypertension, diabetes, and stroke but less frequently had allergic comorbidities, including allergic rhinitis, allergic conjunctivitis, and hay fever; the prevalence of atopy was not different. The use of inhaled corticosteroids and long-acting β_2 agonists was equivalent; however, long-acting muscarinic antagonists were significantly used more

frequently and leukotriene receptor antagonists were significantly less frequently used by ACO patients than by asthma patients without COPD. Oral corticosteroids and biological drugs for asthma were used comparably. ACO patients had significantly higher asthma control questionnaire scores than asthma patients without COPD. For the 121 ACO patients and 472 asthma patients without COPD, the rates of asthma exacerbation and admission were comparable.

Comparison of blood test and serum biomarker levels between ACO and asthma patients without COPD

The comparison of the blood test results and biomarker levels is shown in Table 2. The blood tests revealed that white blood cells, eosinophils, and neutrophils were significantly predominant in ACO patients relative to asthma patients without COPD. In addition, the red blood cell count and hemoglobin levels were significantly higher in ACO patients than in asthma patients without COPD. The serum total IgE levels were also significantly higher in ACO patients than in asthma patients without COPD. The biomarkers of type 2 and non-type 2 inflammation were significantly higher in ACO patients than in asthma patients without COPD: the biomarkers included eotaxin/CCL11, IL1-RA, IL-18, IP-10/CXCL10, MCP-1/CCL2, MMP1, MMP3, MMP8, periostin, ST2/IL-1R4, TARC/CCL17, TIMP1, and YKL-40/CHI3L1. Conversely, the leptin levels were significantly lower in the ACO group than in the asthma group.

ROC curve analysis for biomarkers to discriminate ACO patients from asthma patients

ROC curve analysis of the serum biomarker levels was used to distinguish ACO patients from asthma patients. The area under the curve (AUC), optimal cut-off point determined using the Youden Index, sensitivities, and specificities are shown in Table 3. Among the biomarkers, YKL-40/CHI3L1 showed the highest AUC (0.666, 95% confidence interval [CI]: 0.613–0.714) followed by MMP3 (0.661, 95% CI: 0.608–0.710), IL-1RA (0.660, 95% CI: 0.606–0.709), MMP8 (0.625, 95% CI: 0.569–0.678) and TARC/CCL17 (0.605, 95% CI: 0.550–0.658).

	ACO patients (n = 125)	asthma patients without COPD (n = 514)	*P*-value
Age (years), median (IQR)	73 (66–77)	65 (50–74)	<0.001
Sex male, n (%)	96 (76.8)	167 (32.5)	<0.001
BMI (kg/cm^2), median (IQR)	23.2 (21.1–26.0)	23.5 (21.0–26.4)	0.838
Respiratory function, median (IQR)			
FEV1 (mL)	1620 (1225–2075)	2070 (1540–2640)	<0.001
FVC (mL)	2930 (2405–3665)	2735 (2227.5–3390)	0.146
FEV1/FVC (%)	57 (49–65)	75 (68–82)	<0.001
%FEV1 (%)[a]	71 (54.5–85.5)	97.5 (81.8–111)	<0.001
Self-reported Comorbidity, n (%)			
GERD	24 (19.2)	107 (20.8)	0.688
SAS	15 (12.0)	44 (8.6)	0.234
Hypertension	60 (48.0)	155 (30.2)	<0.001
Diabetes	21 (16.8)	53 (10.3)	0.042
Heart Disease	9 (7.2)	32 (6.2)	0.690
Stroke	6 (4.8)	5 (1.0)	0.003
Arthritis	5 (4.0)	30 (5.8)	0.418
Osteoporosis	8 (6.4)	53 (10.3)	0.182
Mental Disorder	10 (8.1)	68 (14.1)	0.075
Sinusitis	59 (47.2)	230 (45.3)	0.699
Allergic comorbidities	62 (49.6)	373 (72.6)	<0.001
Allergic Rhinitis	23 (18.4)	162 (31.5)	0.004
Allergic Conjunctivitis	3 (2.4)	65 (12.7)	0.001
Atopic Dermatitis	7 (5.6)	52 (10.3)	0.105
Hay Fever	28 (22.4)	211 (41.1)	<0.001
Food Allergy	7 (5.6)	48 (9.3)	0.181
Drug Allergy	10 (8.0)	67 (13.0)	0.121
Urticaria	8 (6.4)	60 (11.7)	0.086
Atopy[b]	73 (58.4)	316 (61.5)	0.527
Treatment, n (%)			
ICS	124 (99.2)	508 (98.8)	0.724
LABA	121 (96.8)	483 (93.9)	0.212
LTRA	91 (72.8)	421 (82.9)	0.022
Theophylline	62 (49.6)	225 (43.8)	0.240
LAMA	61 (49.2)	98 (19.1)	<0.001
Anti-allergic	32 (25.8)	164 (31.9)	0.186
Omalizumab	8 (6.5)	34 (6.7)	0.952
Mepolizumab	4 (3.3)	28 (5.5)	0.311
OCS	11 (8.8)	69 (13.4)	0.161
ACQ SUM6, median (IQR)	1.00 (0.33–1.67)	0.67 (0.17–1.33)	0.028
Patients with exacerbations, n (%)	38 (31.4)	152 (32.2)	0.867
Number of exacerbations, median (IQR)	0 (0–1)	0 (0–1)	0.727
Patients with admissions, n (%)	9 (7.4)	30 (6.4)	0.668

Table 1. Patient characteristics of ACO and asthma patients without COPD. *Abbreviations: ACQ, asthma control questionnaire; BMI, body mass index; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; GERD, gastroesophageal reflux disease; ICS, inhaled corticosteroid; IQR, interquartile range; LABA, long-acting β$_2$ agonist; LAMA, long-acting muscarinic antagonist; LTRA, leukotriene receptor antagonist; OCS, oral corticosteroid; SAS, sleep apnea syndrome. [a]%FEV1 was calculated by dividing FEV1 by predicted FEV1. [b]Specific IgE responsiveness to common inhaled allergens*

	ACO patients (n = 125)	asthma patients without COPD (n = 514)	P-value
WBC (/μL)	6750 (5600–8200)	6200 (5200–7300)	0.004
Eosinophils (/μL)	213 (78–422)	162 (66–309)	0.033
Neutrophils (/μL)	3908 (2991–5009)	3596 (2842–4488)	0.028
RBC ($\times 10^4$/μL)	467 (436-494)	456 (427-487)	0.041
Hb (g/dL)	14.5 (13.4-15.3)	13.8 (12.9-14.7)	<0.001
PLT ($\times 10^4$/μL)	25.0 (21.3-29.6)	25.9 (22.2-30.5)	0.067
IgE (IU/mL)	243.5 (69.7-764.8)	154.5 (55.1-401.5)	0.008
Biomarkers			
Eotaxin/CCL11 (pg/mL)	169.2 (121.6-225.1)	147.0 (100.0-209.2)	0.010
IL-1RA (pg/mL)	1435.9 (1150.2-1745.6)	1169.2 (949.8-1473.0)	<0.001
IL-2 (pg/mL)	32.9 (9-81.8)	38.2 (9-116.3)	0.208
IL-4 (pg/mL)	15.7 (5-49.7)	27.6 (5-62.4)	0.079
IL-7 (pg/mL)	15.1 (11.2-20.1)	15.2 (11.4-19.8)	0.747
IL-8 (pg/mL)	10.9 (8.3-14.4)	9.9 (6.7-15.0)	0.084
IL-18 (pg/mL)	180.4 (144.3-236.3)	167.6 (122.5-228.1)	0.039
IP-10/CXCL10 (pg/mL)	18.7 (14-23.5)	16.4 (12.5-21.8)	0.016
Leptin (ng/mL)	17.8 (11.2-27.1)	21.7 (12.6-40.6)	0.006
MCP-1/CCL2 (pg/mL)	394.2 (309.2-477.5)	367.9 (293.8-448.3)	0.043
MIP1α/CCL3 (pg/mL)	63.8 (33-103.6)	72.9 (33.0-112.9)	0.432
MIP1β/CCL4 (pg/mL)	194.0 (151.9-272.4)	190.0 (144.4-272.0)	0.687
MMP1 (pg/mL)	4436.2 (2885.8-6545.4)	3715.4 (2552.5-5696.2)	0.005
MMP2 (ng/mL)	258.3 (208.3-311.3)	261.0 (215.9-303.5)	0.972
MMP3 (ng/mL)	27.6 (19.2-41.6)	18.8 (13.4-31.0)	<0.001
MMP8 (pg/mL)	1442.5 (887.8-2382.1)	1061.3 (636.4-1633)	<0.001
Periostin (ng/mL)	344.6 (273.4-514.7)	316.7 (240.8-437.5)	0.008
PDGF-BB (pg/mL)	7493.7 (5625.2-9451.4)	7197.1 (5320.4-9637.7)	0.656
RANTES/CCL5 (ng/mL)	28.0 (21.0-43.1)	31.4 (22.2-41.9)	0.520
ST2/IL-1R4 (pg/mL)	8643.4 (6655.2-11717.3)	7399.1 (5497.1-10388.5)	0.001
TARC/CCL17 (pg/mL)	719.3 (504.1-1117.9)	575.5 (375.7-908.0)	<0.001
TIMP1 (ng/mL)	141.3 (123.5-161.6)	132.0 (116.4-150.9)	0.001
TGF-β (ng/mL)	17.9 (15.1-22.9)	18.0 (14.8-21.5)	0.684
YKL-40/CHI3L1 (ng/mL)	82.8 (44.9-143.0)	44.9 (25.4-84.0)	<0.001

Table 2. Blood test results and biomarker levels in ACO and asthma patients without COPD. *Each data represent median (interquartile range (IQR)). Abbreviations: WBC, White Blood Cell; RBC, Red Blood Cell; Hb, hemoglobin; PLT, platelet*

Comparison of serum biomarker levels and clinical factors in ACO patients and asthma patients without COPD among older and non-older patients

As discrimination of ACO can be particularly challenging among older patients, the blood data including some serum biomarker levels which showed high AUC were analyzed using age-stratification, with 65 years as the cut-off point (Table 4). Of the 639 patients, 362 (56.7%) were aged ≥65 years, and 98 (27.1%) were diagnosed with ACO. Of the remaining 277 patients aged <65 years, 27 (9.7%) were diagnosed with ACO. The white blood cell and neutrophil counts were significantly higher only for the patients aged ≥65 years and the serum total IgE levels were significantly higher only for those aged <65 years among ACO patients. Compared with asthma patients without COPD, only the serum MMP3 and IL-1RA levels among all measured biomarkers were significantly higher for both the <65 and ≥ 65 age groups of ACO patients. Among

	AUC (95% CI)	Optimal cut off point	Sensitivity/specificity
YKL-40/CHI3L1 (ng/mL)	0.666 (0.613–0.714)	49.3	72.8%/53.9%
MMP3 (ng/mL)	0.661 (0.608–0.710)	19.8	74.4%/53.3%
IL-1RA (pg/mL)	0.660 (0.606–0.709)	1120.4	82.4%/44.9%
MMP8 (pg/mL)	0.625 (0.569–0.678)	1227.5	61.6%/61.1%
TARC/CCL17 (pg/mL)	0.605 (0.550–0.658)	664.9	61.6%/58.6%
ST2/IL-1R4 (pg/mL)	0.599 (0.544–0.652)	7980.7	60.8%/55.8%
TIMP1 (ng/mL)	0.595 (0.537–0.650)	145.8	46.4%/69.5%
MMP1 (pg/mL)	0.581 (0.526–0.634)	4704.3	47.2%/66.5%
Leptin (ng/mL)	0.579 (0.525–0.632)	29.1	81.6%/37.9%
Periostin (ng/mL)	0.576 (0.520–0.630)	261.3	82.4%/31.3%
Eotaxin/CCL11 (pg/mL)	0.575 (0.520–0.627)	106.6	86.4%/28.6%
IP-10/CXCL10 (pg/mL)	0.569 (0.512–0.625)	18.3	53.6%/60.7%
IL-18 (pg/mL)	0.559 (0.503–0.615)	150.9	73.6%/42.4%
MCP-1/CCL2 (pg/mL)	0.558 (0.502–0.614)	383.9	56.8%/57.4%

Table 3. Receiver operating characteristics (ROC) curve analysis for biomarkers to discriminate ACO patients from asthma patients. *ROC analysis to compare the accuracy of each biomarker to distinguish ACO from asthma. Abbreviations: AUC, Area Under the Curve; CI, Confidence interval*

older patients, the levels of YKL-40/CHI3L1 were not different between the 2 groups. In addition to these biomarkers, airflow obstruction was more severe in the ACO patients than in asthma patients without COPD, regardless of age. The rates of exacerbation and admission were comparable for both groups (data not shown).

Multivariable logistic regression analysis of candidate biomarkers

To evaluate the effect of age and sex on biomarkers that discriminate ACO patients from asthma patients, multivariable analysis was performed for YKL-40/CHI3L1, MMP3, and IL-1RA (Table 5). The serum level of MMP3 (OR: 1.201; 95%CI: 1.004–1.437; per 20 ng/mL) and IL-1RA (OR: 1.897; 95%CI: 1.229–2.926; per 1000 pg/mL) were found to be independent and significantly higher in ACO patients. However, the serum levels of YKL-40/CHI3L1 (OR: 1.103; 95% CI: 0.943–1.290; per 100 ng/mL) did not show statistical significance. To eliminate the effects of corticosteroids and biologics, the patients using oral corticosteroids or biologics were excluded.

However, the results were the same: univariate analysis showed that the serum YKL-40/CHI3L1, MMP3, and IL-1RA levels were significantly higher in ACO patients than in asthma patients without COPD, but multivariate analysis showed that only MMP3 and IL-1RA remained significantly higher in patients with ACO (data not shown).

Correlations between age and serum YKL-40/ CHI3L1, MMP3, and IL-1RA levels

Correlation analysis was used to determine the effect of age on the serum biomarker levels (Fig. 2). The serum YKL-40/CHI3L1 levels were strongly and positively correlated with age ($\rho = 0.568$, $P < 0.001$) (Fig. 2A). In contrast, the correlations between the serum MMP3 ($\rho = 0.123$, $P = 0.002$) and IL-1RA ($\rho = 0.116$, $P = 0.003$) levels and age were very weak (Fig. 2B and C).

Correlations between %FEV1 and serum YKL-40/ CHI3L1, MMP3, and IL-1RA levels

Finally, the correlations between %FEV1 and the serum biomarker levels were determined, as

	≧65 years old			<65 years old		
	ACO patients (n = 98)	asthma patients without COPD (n = 264)	p-value	ACO patients (n = 27)	asthma patients without COPD (n = 250)	p-value
Age (years)	75 (70–79)	73.5 (69–79)	0.252	60 (55–63)	50 (41–58)	<0.001
Sex male, n (%)	75 (76.5)	79 (29.9)	<0.001	21 (77.8)	88 (35.2)	<0.001
BMI (kg/cm^2)	23.0 (21.1–25.5)	23.4 (21–25.9)	0.580	25.3 (22.9–27.8)	23.7 (20.8–27.0)	0.225
Respiratory function, median (IQR)						
FEV1 (mL)	1550 (1155–1953)	1670 (1330–2110)	0.048	1800 (1449–2390)	2520 (2068–3055)	<0.001
FVC (mL)	2830 (2268–3423)	2335 (2023–2830)	<0.001	3220 (2760–4010)	3155 (2730–3843)	0.627
FEV1/FVC (%)	56 (49–65)	72 (65–78)	<0.001	61 (44–66)	79 (71–85)	<0.001
%FEV1 (%)[a]	71 (52–87.5)	97 (80–114)	<0.001	69 (55–83)	98 (85–109)	<0.001
WBC (/μL)	6800 (5600–8150)	6100 (5200–7250)	0.004	6500 (5350–8650)	6400 (5200–7500)	0.355
Eosinophils (/μL)	216 (77–418)	174 (64–299)	0.104	194 (82–455)	154 (69–316)	0.178
Nuetrophils (/μL)	4002 (3023–5067)	3604 (2914–4428)	0.025	3630 (2792–5094)	3570 (2749–5094)	0.727
IgE (IU/mL)	192.0 (63.2–687.5)	149.0 (55–379.5)	0.105	559.0 (145.0–1515.0)	156.0 (54.7–450.0)	0.003
Biomarkers						
YKL-40/CHI3L1 (ng/mL)	88.2 (46.7–145.4)	70.6 (42.1–126.7)	0.147	62.1 (30.9–114.7)	29.8 (17.5–46.6)	<0.001
MMP3 (ng/mL)	28.0 (19.6–41.1)	19.0 (13.9–33.4)	<0.001	27.4 (17.9–46.0)	18.4 (13.1–28.2)	0.009
IL-1RA (pg/mL)	1443.3 (1170.3–1744)	1176.7 (972.7–1491.1)	<0.001	1359.5 (1123.9–1767.8)	1149.4 (927.0–1455.0)	0.007
MMP8 (pg/mL)	1492.5 (878.2–2434.7)	1044.5 (605.7–1493.8)	<0.001	1168.0 (954.8–1920.5)	1066.6 (669.4–1782.6)	0.498
TARC/CCL17 (pg/mL)	731.5 (507.5–1105.2)	605 (395.9–909.0)	0.004	682.2 (469.8–1196.8)	522.2 (359.0–897.1)	0.113

Table 4. Clinical characteristics and serum biomarker levels which showed high AUCs for distinguishing ACO patients and asthma patients without COPD among older and non-older patients. *Continuous variables are presented as medians (IQR). Abbreviations: BMI, body mass index; WBC, White Blood Cell. [a]%FEV1 was calculated by dividing FEV1 by predicted FEV1*

YKL-40/CHI3L1	odds ratio	95% CI	P-value
Age (per 10 years)	1.908	1.559–2.335	<0.001
Sex: male	7.858	4.857–12.714	<0.001
YKL-40/CHI3L1 (per 100 ng/mL)	1.103	0.943–1.290	0.235
MMP3	odds ratio	95% CI	P-value
Age (per 10 years)	1.908	1.559–2.335	<0.001
Sex, male	6.906	4.216–11.315	<0.001
MMP3 (per 20 ng/mL)	1.201	1.004–1.437	0.038
IL-1RA	odds ratio	95% CI	P-value
Age (per 10 years)	1.867	1.526–2.284	<0.001
Sex: male	6.863	4.222–11.156	<0.001
IL-1RA (per 1000 pg/mL)	1.897	1.229–2.926	0.004

Table 5. Multivariable logistic regression analysis on YKL-40/CHI3L1, MMP3, and IL-1RA. *Abbreviations: CI, Confidence interval*
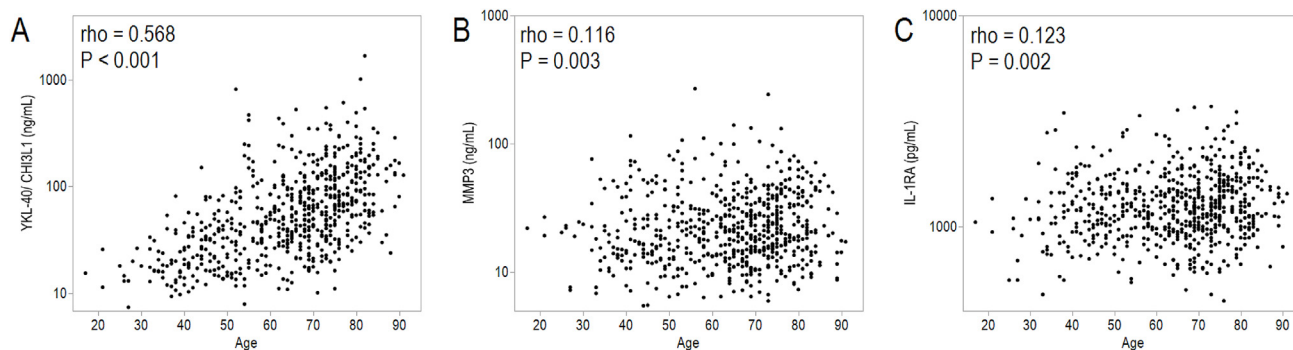
Fig. 2 Correlations between age and serum YKL-40/CHI3L1 (A), MMP-3 (B), and IL-1RA (C) levels. Serum biomarker values are expressed logarithmically

shown in Fig. 3. The serum IL-1RA ($\rho = -0.202$, $P < 0.001$) levels showed weak but significant negative correlations with FEV1% (Fig. 3A–C). The blood eosinophil count is reported to be negatively correlated with respiratory functions;[23] however, it was not correlated with the YKL-40/CHI3L1, MMP3, and IL-1RA levels in this study (data not shown).

DISCUSSION

This study shows the characteristics and serum biomarkers of ACO and asthma in Japan. ACO showed a trend of higher predominance among males and older adults and was associated with higher prevalence of comorbid hypertension, diabetes, and stroke. Serum MMP-3 and IL-1RA levels may be biomarkers to discriminate ACO from asthma without COPD, in older and young asthma patients.

In this study, 19.6% of patients with asthma were diagnosed with ACO. The reported proportions of asthma patients with ACO range from 13.3 to 61.0%.[24] This wide range may be due to differences in age, sex, race, and the definitions of ACO for the cohorts. Old age has been reported as the greatest contributor to this disparity,[25,26] which is consistent with the results of the present study involving the old age cohort. In this study, ACO patients were older and predominantly male, which is consistent with previous reports.[25,26] In Japan and other places
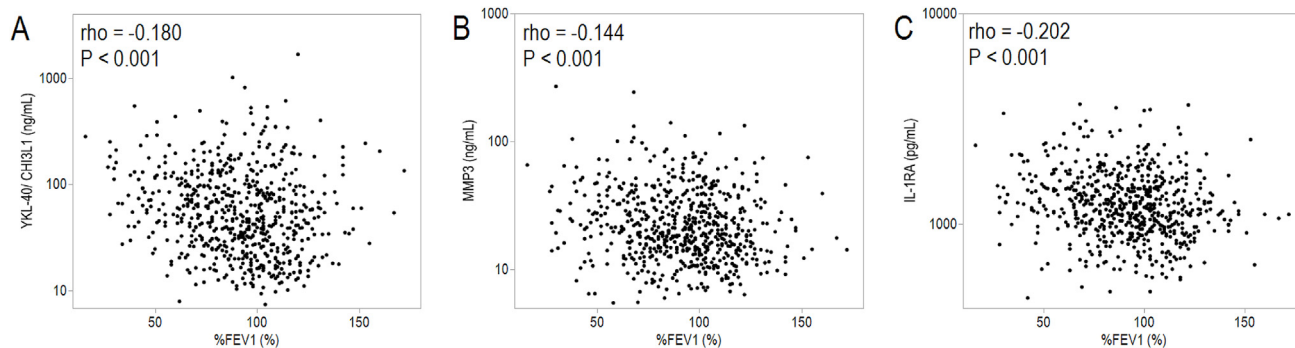
Fig. 3 Correlations between %FEV1 (FEV1/predicted FEV1) and serum YKL-40/CHI3L1 (A), MMP-3 (B), and IL-1RA (C) levels. The serum biomarker levels are expressed logarithmically. Abbreviations: FEV1, forced expiratory volume in 1 s

in the world, men and older patients tend to smoke for longer periods, resulting in a higher incidence of COPD.[27]

COPD is a chronic pulmonary disease characterized by emphysema and airway damage due to lung inflammation, but systemic inflammation is also found in COPD patients, and is associated with complications and other comorbidities. The majority of COPD patients have several comorbidities, some of which are associated with a high risk of mortality.[28,29] Hypertension and diabetes mellitus were more prevalent in ACO patients in the present study. They are major comorbidities of COPD,[28,29] and they have been reported to be common among ACO patients as well.[30] The prevalence of stroke was also higher among ACO patients in the present study, which is also consistent with a previous report.[31] On the other hand, allergic conditions, such as allergic rhinitis, allergic conjunctivitis, hay fever, and urticaria, were more common among control asthma patients in the present study, although the blood eosinophils and serum IgE levels were higher for the patients with ACO. Since allergic sensitization and asthma are closely related and there is little or no relationship between allergic sensitization and COPD, the higher prevalence of allergic diseases among asthma patients without COPD seemed reasonable. The mechanisms underlying the increase in the eosinophil and IgE in ACO patients remain to be determined; however, smoking increases the total serum IgE level.[32,33] In a study of adult asthma patients in Japan, smoking also contributed to an increase in the blood eosinophil count.[32] In a cluster analysis of Japanese patients with severe asthma, smoking

patients had 2 main clusters, which were the high and low blood eosinophil count and IgE level clusters.[34] Considering that the blood neutrophil counts were also higher in the ACO patients, ACO patients in this study may be grouped into 2: high and low eosinophil count and IgE level groups, which are characterized by different inflammatory pathways.

Biomarkers, such as fractional exhaled nitric oxide levels, blood eosinophil count, and blood IgE levels have been reported to be useful in distinguishing ACO from COPD.[35,36] However, useful biomarker(s) for distinguishing ACO from asthma are still needed. YKL-40/CHI3L1 is secreted by various cells, such as macrophages, neutrophils, monocytes, vascular smooth muscle cells, synovial cells, and chondrocytes, during inflammation and tissue remodeling.[37,38] In asthma patients, the serum YKL-40/CHI3L1 level is correlated with the severity of asthma and inversely correlated with the forced expiratory volume in 1 s (FEV1).[39] Additionally, the serum YKL-40/CHI3L1 level is increased in COPD patients,[15-17] and some studies have shown that the serum YKL-40/CHI3L1 level is higher in ACO patients than in asthma patients.[15,16] However, the serum YKL-40/CHI3L1 level is influenced by age; therefore, adjustments for age are necessary.[15,40] In line with this, the serum YKL-40/CHI3L1 was positively correlated with age in this study. Furthermore, when the data of only patients aged ≥65 years were analyzed, the significant difference between ACO patients and asthma patients without COPD was not significant, and multivariable analysis also showed that YKL-40/CHI3L1 was not an independent factor for discriminating ACO patients from asthma.

In the present study, the levels of several inflammatory cytokines were elevated in the serum of ACO patients. For instance, the levels of chemokines related to the recruitment of macrophages and neutrophils, including IL-18,[41] IP-10/CXCL10,[42] MCP-1/CCL2,[43] and periostin[44] were higher in ACO patients. Macrophages and neutrophils are critical cells in COPD pathology. However, among these biomarkers, only the serum MMP3 and IL-1RA levels were significantly higher in the ACO patients than in the controls among patients aged both ≥65 and < 65 years. Furthermore, serum MMP3 and IL-1RA levels were predictors of ACO patients independent of age and sex. In COPD lungs, the protease-antiprotease imbalance transitions to protease dominance, and matrix metalloproteinases (MMPs) play important roles in the pathogenesis of emphysema.[45,46] Among MMPs, MMP12 causes elastin and collagen degeneration in emphysema, and MMP9 contributes to small airway thickening.[46] In a clinical observation, the salivary MMP8, MMP9, and MMP12 levels were increased in COPD patients.[47] The MMP8 and MMP9 levels in bronchoalveolar lavage fluid were also elevated among current smokers with emphysema.[48] The blood test showed elevated MMP2, MMP3, and MMP8 concentrations in COPD patients.[49-51] MMP3 activates MMP1 and MMP9,[52] and it may directly or indirectly be involved in COPD. In this study, serum MMP3 levels were higher in ACO patients, regardless of age, suggesting usefulness of serum MMP3 in determining ACO in asthma patients.

IL-1RA, which was another biomarker effective for distinguishing ACO from asthma in the present study, is a naturally occurring cytokine that exerts anti-inflammatory effects by inhibiting the IL-1 inflammatory pathway.[53,54] It is induced to suppress the effects of IL-1α and IL-1β in COPD. A study reported that the IL-1α, IL-1β, and IL-1RA levels in the sputum were higher in COPD patients who smoked,[55] while the serum IL-1RA were higher in COPD than in asthma patients despite the equivalent IL-1β levels.[56] The elevated serum IL-1RA levels in ACO patients may support the previous suggestions that elevated IL-1RA levels reflect severe inflammation in COPD. The serum IL-1RA levels were found to be independently associated with ACO and showed minimal correlation with age. Additionally, there was a minimal but significant negative correlation between IL-1RA and %FEV1, suggesting that IL-1RA may also be a candidate biomarker for distinguishing ACO from asthma patients.

This study had some limitations. First and most importantly, the designation of ACO was based on a combination of self-reported history of COPD/emphysema, smoking history, and the presence of obstructive disorders, rather than solely relying on post-bronchodilator measurements. Since the primary study, NHOM-Asthma, recruited asthma patients, chest radiological examinations and post-bronchodilator pulmonary function tests were not necessary, and clinical examinations may not have been enough for assessing COPD. However, the patients who had not undergone an airway reversibility test were continuously on asthma medications including bronchodilators, so detected airflow obstruction under asthma medications may well be a fixed change. In addition, it should be noted that the association between smoking, airflow obstruction, and COPD is very strong even in asthma, and smoking has been shown to increase the risk of adult airway obstruction in asthma with disease onset after the age of 10 years.[57] Secondly, comorbidities, including COPD and smoking history, were analyzed based on self-reported questionnaire responses, which may affect the credibility of the study results. Thirdly, the patients who participated in this study were from national hospitals in Japan and not primary medical institutions, which may have resulted in selection bias. Older patients who could not visit national hospitals due to their disabilities or younger patients who could not visit due to their busy schedules may not have been included. Lastly, this study is a post-hoc analysis of a single cohort that used the data obtained from a previous study, and healthy controls were not included. A larger prospective study with thorough examinations, including sputum markers, of both asthma and COPD is needed to validate the results.

In conclusion, this study suggests that MMP3 and IL-1RA may be serum biomarkers for distinguishing ACO and asthmatic patients. In addition, they may facilitate the identification of a new pathological background of ACO.

Abbreviations

ACO, asthma-COPD overlap; ACOS, asthma-COPD overlap syndrome; ACQ, asthma control questionnaire; AUC, area under the curve; COPD, Chronic Obstructive Pulmonary Disease; FEV1, forced expiratory volume in the first second; ROC, receiver operating characteristics.

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Data availability

Not available.

Author contributions

Conceptualization, K.T. and M.S.; methodology, K.T. and M.S.; software, K.T.; validation, K.T. and M.S.; formal analysis, K.T.; investigation, K.T.; data curation, H.T., N.O., Y.F., N.K., M.T., M.I., M.A., and K.O.; writing–original draft preparation, K.T.; writing–review and editing, K.T., M.S., H.T., N.O., Y.F., N.K., M.T., M.I., M.A., and K.O.; visualization, K.T.; supervision, H.T., N.O., Y.F., N.K., M.T., M.I., M.A., and K.O.; project administration, M.S., Y.F., M.T., and K.O.

Ethics statement

This study was approved by the Institutional Review Board of the National Hospital Organization Tokyo National Hospital (Approval No. 529). The requirement for informed consent was waived because this study was only based on the data from the previous study.

Consent for publication

All authors have read and agreed to the published version of the manuscript.

Submission declaration

Our manuscript is original, has not been published before, is not currently being considered for publication elsewhere.

Declarations of competing interest

Acknowledgements

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